motors were placed in defined positions along the scaffold. Further, the flexibility of the scaffold could be externally modulated to alter coupling between the different motors. This approach enables considerably more precision in defining the mechanics of these multi-motor systems and experiments using coupled dimeric motors are underway.

While irreversibly binding motors to glass beads is an optimal approach for *in vitro* mechanical experiments and may be a good model for attachment to protein complexes in cells, this arrangement differs quite a bit from the attachment of kinesins to intracellular cargo, such as vesicles, Golgi, and mitochondria. When attached to a membrane, kinesins are free to move laterally in the fluid bilayer, which on one hand reduces the potential mechanical coupling between motors but on the other hand allows dynamic clustering of motors, leading to significant forces. This geometry has been successfully recapitulated *in vitro* by attaching kinesins to giant unilamellar vesicles and characterizing the extraction of membrane nanotubes by the attached motors [10,11]. Importantly, as is predicted for the rigid attachment to cargo, motors with different degrees of processivity show considerably different cooperative dynamics in this system.

Interesting and non-intuitive phenomena are observed when groups of motor proteins are attached to beads, protein scaffolds, and membranes. The power of these *in vitro* systems is the ability to vary experimental parameters and use quantitative models to predict and interpret experimental findings. Ongoing experiments and modeling should lead to important insights regarding transport by groups of motors in cells.

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Candida Biofilms: Is Adhesion Sexy?

The development of *Candida albicans* biofilms requires two types of adhesion molecule — the Als proteins and Hwp1. Mutational analyses have recently revealed that these molecules play complementary roles, and their characteristics suggest that they may have evolved from primitive mating agglutinins.

David R. Soll

In the past decade, bacteriologists, and more recently mycologists, have begun to realize that the microbes they study frequently infect hosts, not as free-living, planktonic organisms, but as multicellular biofilms that form on tissues, prosthetics and catheters [1–3]. Biofilms protect a pathogen from host defenses and antibiotics, and provide it with a degree of spatial stability and autonomy in controlling its own microenvironment. A biofilm utilizes sophisticated intercellular communication systems (such as quorum sensing in bacteria [4]), involves the formation of an extracellular polymeric matrix, depends on adhesion both to substrates and between cells, and can be composed of multiple cell types. Many of these characteristics are shared with tissues of higher eukaryotes, an analogy that evokes the hypothesis that biofilms formed by microbes may represent the first steps in the evolution of multicellularity in higher eukaryotes.

Candida albicans, the most pervasive fungal pathogen that colonizes humans, also forms biofilms on tissues, prosthetics, and catheters [2,3]. Although usually associated exclusively with host colonization, the formation of a biofilm by *C. albicans* may have preceded host colonization in the evolution of the organism, perhaps as a mechanism to protect cell propagation in a hostile environment, such as in soil or on a rock at the edge of a pond. In the formation of a *C. albicans* biofilm (Figure 1), cells first adhere to the substratum. This results in the formation of a confluent basal layer of cells that divide and produce compartmentalized hyphae, long tubular projections that intertwine in the upper region of the biofilm (Figure 1). Cells in the developing biofilm release a stable extracellular matrix of polymeric substances. Adhesion must play a major role throughout the development of a *C. albicans* biofilm: firstly, it must secure cells to the substratum and may bind them to one another in the formation of a basal layer, the first step in biofilm formation(s); and secondly, it may bind hyphae to each other, thus stabilizing the maturing biofilm. In both bacteria and fungi, our understanding of the adhesive forces involved in biofilm formation is rudimentary. Elucidating such adhesive mechanisms would be extremely useful in developing new

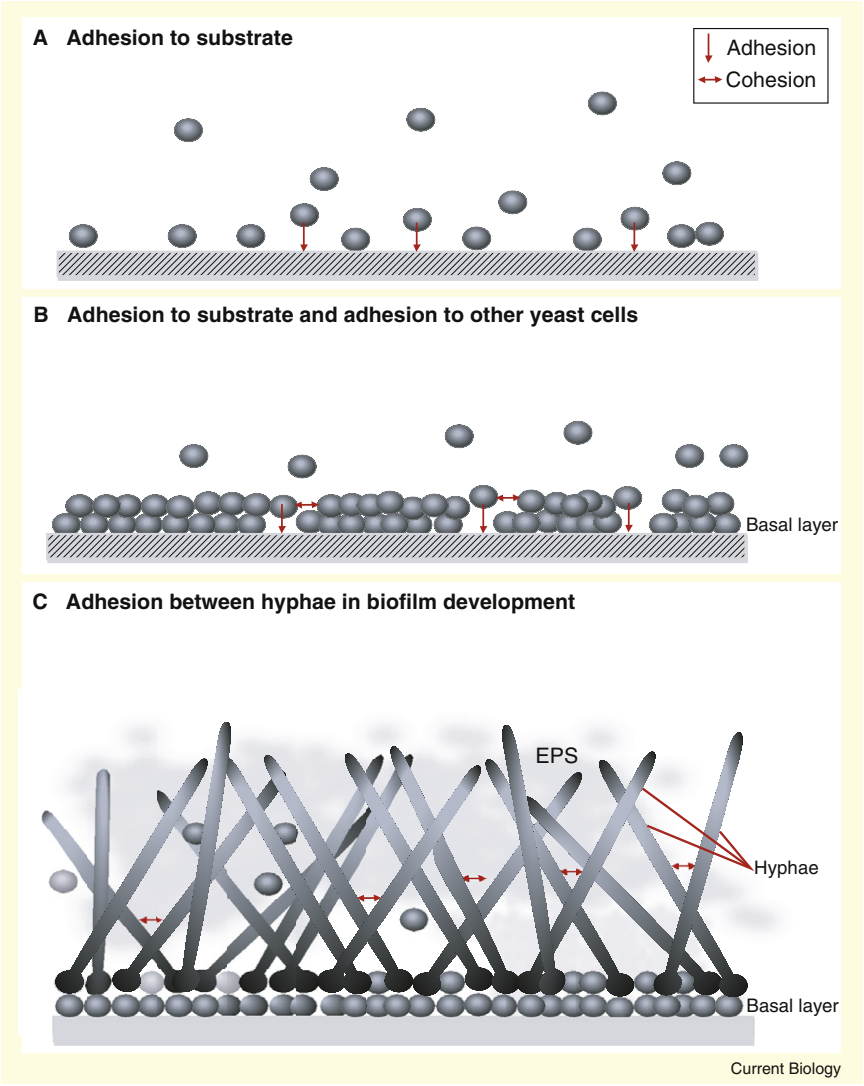

Figure 1. The role of adhesion in biofilm development in *C. albicans*.

C. albicans forms multicellular biofilms on a variety of surfaces including host tissues, prosthetics (such as dentures) and catheters. This process has been studied on flat surfaces *in vitro* and in venus catheters in a rat infection model *in vivo*. (A,B) In the initial development of a biofilm *in vitro*, yeast cells bind to a surface and to one another to form a basal layer of cells [19]. Adhesion is necessary for surface binding and for binding of one cell to another (cohesion). (C) From this basal layer of yeast cells, hyphae are formed. Hyphae are tubular projections that are compartmentalized into cellular units with nuclei. Adhesion between tubes serves to stabilize the biofilm. From hyphae, additional yeast cells can form. Between the tubes, an extracellular polymeric matrix forms. A biofilm provides a microenvironment that protects cells from being washed away in fluid areas, like the mouth, gut and vagina, provides resistance to antibiotics and white blood cell invasion, and allows cells to condition their microenvironment. Understanding the mechanisms that are employed to build and maintain a biofilm, like adhesion, is crucial for the development of therapeutic strategies to disrupt them in the treatment of candidiasis. EPS, extracellular polymeric substances.

drugs and therapies for many of the diseases these pathogens cause.

In a recent issue of *Current Biology*, Nobile *et al.* [5] present evidence that two types of surface molecule, the Als proteins and Hwp1, both previously implicated in adhesion and biofilm formation, play complementary roles in biofilm development in *C. albicans*, suggesting that they may interact to form heterotypic bonds between adjacent cell surfaces. Based on the observations that the Als proteins share structural features with the α mating agglutinin that is expressed on the surface of α cells of *Saccharomyces cerevisiae*, that Hwp1 is selectively expressed on conjugation tubes of mating type a but not α cells of *C. albicans*, and that the two types of molecule play complementary roles in biofilm development, Nobile *et al.* [5] have put forward the novel hypothesis that a heterotypic Als–Hwp1 adhesion system may have evolved in biofilm formation from ancestral mating agglutinins.

ALS genes were initially demonstrated to be upregulated during biofilm formation [2,6–8]. A null mutant of *ALS3*, one of eight *ALS* genes [8], produced fragile biofilms with aberrant hyphal architecture. It was further demonstrated that a null mutant of the gene encoding the transcription factor Bcr1 was defective in both biofilm formation and *ALS3* expression, again implicating *ALS3* in biofilm development [9,10]. Overexpression of *ALS3*, or *ALS1*, another member of the *ALS*-gene family that is closely related to *ALS3*, rescued the *bcr1* defect in biofilm development, suggesting for the first time that the two *ALS* genes were not only necessary for normal biofilm formation, but may have redundant functions [10]. In addition to *ALS3* and *ALS1*, overexpression of a third, unrelated gene, *HWP1* [11], also improved biofilm development in a *bcr1* background in which *ALS1* and *ALS3* were not upregulated [10].

The recent results of Nobile *et al.* [5] indicate the following: firstly, Als1 and Als3 together are necessary for normal biofilm development in an *in vivo* catheter model and appear to have overlapping, or redundant, functions; secondly, overexpression of a subset of related *ALS* genes — *ALS5*, *ALS6*, *ALS7* and *ALS9* — can partially or completely substitute for the absence of both *ALS1* and *ALS3* by facilitating biofilm development in the *in vivo* model, but two other *ALS* genes, *ALS2* and *ALS4*, cannot; thirdly, all of the *ALS* genes can substitute for *ALS1* and *ALS3* in an *in vitro* model; and finally, *ALS1*/*ALS3* and *HWP1* function in a complementary fashion at the cellular level in biofilm development in both models. The last of these results suggests that *ALS1*/*ALS3* may undergo a heterotypic interaction with Hwp1 between the surfaces of adjacent cells to maintain the integrity of a developing biofilm. There are also indications that this cooperative interaction may be necessary for the adhesion of yeast cells to the substratum. But functional cooperation in the formation of

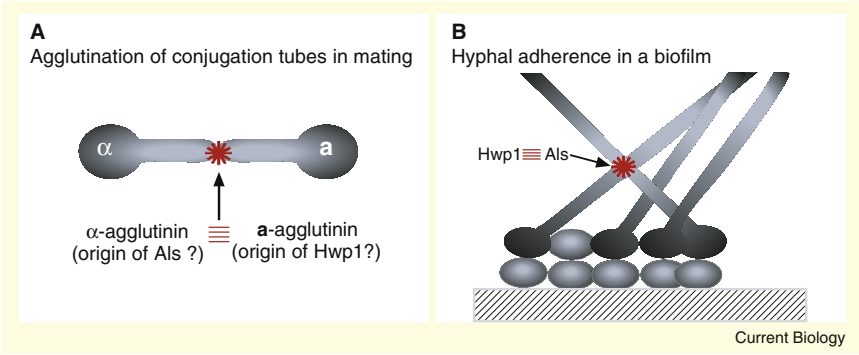

Figure 2. A proposed link between mating agglutination and biofilm formation.

Nobile *et al.* (6) hypothesize that an Als–Hwp1 adhesion system that binds cells in a *C. albicans* biofilm evolved from a primitive mating agglutination system. (A) In the *C. albicans* mating process, mating projections extend from cells of opposite mating types (**a** and α) and, through chemotropism, find each other. The tips of these projections, known as conjugation tubes, then bind to one another to facilitate cell fusion, so that the nuclei of the two cell types can fuse. Conjugation tubes share both morphological features and a number of similarly upregulated genes with growing hyphae. (B) Proposed heterotypic interaction between Als and Hwp1 in hyphal adherence in a biofilm. Nobile *et al.* [5] suggest that Als proteins may have evolved from an α-agglutinin and Hwp1 from an **a**-agglutinin.

a biofilm does not constitute direct proof of a physical interaction between Als1/3 and Hwp1. Additional experiments, using methods such as fluorescence resonance energy transfer (FRET), will be required to prove this point. Moreover, the possible effects on the proposed interaction of other surface molecules, such as Eap1, Sun41 and members of the CFEM family, need to be considered.

What is so intriguing about the findings of Nobile *et al.* [5] is the implication that biofilm development may have its roots in the mating system of an ancestor of modern *C. albicans*. The amino-terminal regions of the Als proteins contain immunoglobulin-like folds similar to those in the amino-terminal region of the *S. cerevisiae* α-cell mating agglutinin, Sag1 [12,13], but the residues critical for binding in Sag1 are not conserved, suggesting a different specificity. Although Hwp1 does not share obvious characteristics with the **a**-cell mating agglutinin, it has a complementary function with Als1/3 and is differentially expressed on **a**/**a**, but not α/α, conjugation tubes during *C. albicans* mating [14]. From these observations, Nobile *et al.* [5] hypothesize that the Als and Hwp1 components of the suggested heterotypic adhesion system may have evolved from α and **a** agglutinins, respectively, of an ancestral mating agglutination system (Figure 2). The evolution of this biofilm agglutination system would have had to occur without compromising the agglutination system that still functions in the mating process of *C. albicans*.

The unique hypothesis proposed by Nobile *et al.* [5] represents the second time that a link between mating and biofilm development has been proposed. It was previously demonstrated that a unique signaling system exists between opaque and white cells of the *C. albicans* white–opaque switching system [15]. Mating-competent opaque cells, through the release of mating pheromone, appear to signal to mating-incompetent white cells to form thicker biofilms, which have been shown *in vitro* to facilitate mating. But the relationship between biofilm development and mating may have had even earlier roots. Mating (conjugation) of the bacterium *Escherichia coli* has been shown to occur at frequencies 1,000 times higher in biofilms than under classical plating conditions [16]. Moreover, natural conjugation plasmids induce the development of *E. coli* biofilms [17], and the pilus, which mediates adhesion through a heterotypic interaction with non-pilus surface molecules on cells of the opposite conjugation type, appears to be essential for biofilm induction [18]. Hence, biofilm formation by *E. coli* is facilitated by a heterotypic interaction of surface molecules that function as mating adhesins, and biofilms formed by *E. coli* facilitate mating, two components of a scenario similar to the one unfolding for *C. albicans*. Nobile *et al.* [5], therefore, may not only have uncovered a link between mating agglutination and biofilm formation in the evolution of *C. albicans* virulence, but also a possible link between prokaryotic and lower eukaryotic biofilm development. Does this represent a true evolutionary thread between prokaryotic and lower eukaryotic biofilm development, or parallel evolution? Either way, what seems clear is that adhesion is 'sexy' in biofilm development.

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